Remarks

The specification has been amended by amending the priority claim of the application and to correct misspellings and typographical errors. Claims 1-66 and 80-92 have been canceled without prejudice. Accordingly, claims 67-79 are pending.

Applicants request early and favorable action in the above-identified application.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Line 7 to line 10 on page 1 has been amended as follows:

This application is a divisional application of U.S. Application Number 10/316,127, entitled SYSTEMS AND METHODS FOR TREATING PATIENTS WITH PROCESSED LIPOASPIRATE CELLS, and filed December 9, 2002, which claims the benefit of U.S. Provisional Application Number 60/338,856, entitled BEDSIDE DEVICE, SYSTEM AND USE OF PROCESSED LIPOASPIRATE CELLS AND ADIPODERIVED STEM CELLS, and filed December 7, 2001, the entire contents of which are hereby incorporated by reference.

Line 6 to line 13 on page 4 has been amended as follows:

A number of devices have been developed for harvesting cells from adipose tissue, but these devices can suffer from one or more of inability to optimally accommodate an [asperation] aspiration device for removal of adipose [tisssue] tissue, lack of partial or full automation from the harvesting of adipose tissue phase through the processing of tissue phases, lack of volume capacity greater than 100ml of adipose tissue, lack of a partially or completely closed system from the harvesting of adipose tissue phase through the processing of tissue phases, and lack of [disposabilty] disposability of components to attenuate concomitant risks of cross-contamination of material from one sample to another.

Line 15 to line 27 on page 5 has been amended as follows:

In one embodiment, a method of treating a patient includes steps of: a) providing a tissue removal system; b) removing adipose tissue from a patient using the tissue removal system, the adipose tissue having a concentration of stem cells; c) processing at least a [paet] part of the adipose tissue to obtain a concentration of stem cells other than the concentration of stem cells of

the adipose tissue before processing; and d) administering the stem cells to a patient without removing the stem cells from the tissue removal system before being administered to the patient.

In another embodiment, a method of treating a patient includes: a) providing an adipose tissue removal system; b) removing adipose tissue from a patient using the adipose tissue removal system, the adipose tissue having a concentration of stem cells; c) processing the adipose tissue to increase the concentration of stem cells in the adipose tissue; d) mixing the adipose tissue having the concentrated stem cells with another unit_portion of adipose tissue; and e) administering the adipose tissue with the increased concentration of stem cells to a patient.

Line 17 to line 25 on page 7 has been amended as follows:

Although the disclosure herein refers to certain illustrated embodiments, it is to be understood that these embodiments are presented by way of example and not by way of limitation. The intent of the following detailed description, although discussing exemplary embodiments, is to be construed to cover all modifications, alternatives, and equivalents of the embodiments as may fall within the spirit and scope of the invention as defined by the appended claims. The present invention may be practiced in conjunction with various cell or tissue [seperation] separation techniques that are conventionally used in the art, and only so much of the commonly practiced process steps are included herein as are necessary to provide an understanding of the present invention.

Line 6 to line 17 on page 10 has been amended as follows:

For suction-assisted lipoplastic procedures, adipose tissue is collected by insertion of a cannula into or near an adipose tissue depot present in the patient followed by aspiration of the adipose into a suction device. In one embodiment, a small cannula may be coupled to a syringe, and the adipose tissue may be aspirated using manual force. Using a syringe or other similar device may be desirable to harvest relatively moderate amounts of adipose tissue (e.g., from 0.1 ml to several hundred milliliters of adipose tissue). Procedures employing these relatively small

devices have the advantage that the procedures can be performed with only local anesthesia, as opposed to general anesthesia. Larger volumes of adipose tissue above this range (e.g., greater than several hundred [millilters] milliliters) may require general anesthesia at the discretion of the donor and the person performing the collection procedure. When larger volumes of adipose tissue are desired to be removed, relatively larger cannulas and automated suction devices may be employed in the procedure.

Lines 22 on page 11 to line 17 on page 12 have been amended as follows:

Patients undergoing treatment in accordance with the disclosure herein receive a different concentration of stem cells than other treatments employing adipose tissue or stem cells derived from adipose tissue. Thus, the adipose tissue that is removed from a patient is processed to change the concentration of stem cells that are administered to the patient. In a preferred embodiment of the invention, patients receive a higher concentration of stem cells than the concentration of stem cells typically present in adipose tissue transplants and other similar stem cell based therapies. The concentrated stem cells may be administered in a composition comprising adipo-derived stem cells and/or endothelial precursor cells substantially free from mature adipocytes and connective tissue, or, as another example, the concentrated stem cells may be administered in a composition comprising a unit of adipose tissue with an increased amount of stem cells. A composition of the invention includes a concentration of stem cells that is greater than the concentration of stem cells found in an equivalent unit of non-processed adipose tissue. In certain embodiments, the composition has a cellular component in which at least 0.1% of the cells are stem cells. In other embodiments, the composition has a cellular component in which the stem cells comprise between about 2% and 12% of the cellular component. Higher concentrations_of stem cells, such as up to 100%, are also included in different compositions. The composition may include additional components, such as cell differentiation factors, growth promoters, immunosuppressive agents, or medical devices, as discussed herein. To obtain certain compositions in which the composition primarily contains one type of cell (e.g., adipo-derived stem cells or adipo-derived endothelial precursor cells), any suitable method for

separating the different cell types may be employed, such as the use of cell-specific antibodies that recognize and bind antigens present on either stem cells or endothelial precursor cells.

Line 12 to line 31 on page 13 has been amended as follows:

The intact tissue fragments are then disaggregated using any conventional techniques or methods, including mechanical force (mincing or shear forces), enzymatic digestion with single or combinatorial [protelolytic] proteolytic enzymes, such as collagenase, trypsin, lipase, liberase H1, as disclosed in U.S. Pat. No. 5,952,215, and pepsin, or a combination of mechanical and enzymatic methods. For example, the cellular component of the intact tissue fragments may be disaggregated by methods using collagenase-mediated dissociation of adipose tissue, similar to the methods for collecting microvascular endothelial cells in adipose tissue, as disclosed in U.S. Patent No. 5,372,945. Additional methods using collagenase that may be used in practicing the invention are disclosed in U.S. Patent No. 5,830,714 and 5,952,215, and by Williams, S. K., S. McKenney, et al. (1995). "Collagenase lot selection and purification for adipose tissue digestion." Cell Transplant 4(3): 281-9. Similarly, a neutral protease may be used instead of collagenase, as disclosed in Twentyman, P. R. and J. M. Yuhas (1980). "Use of bacterial neutral protease for disaggregation of mouse tumours and multicellular tumor spheroids." Cancer Lett 9(3): 225-8. Furthermore, methods may employ a combination of enzymes, such as a combination of collagenase and trypsin, as disclosed in Russell, S. W., W. F. Doe, et al. (1976). "Inflammatory cells in solid murine neoplasms. I. Tumor disaggregation and identification of constituent inflammatory cells." Int J Cancer 18(3): 322-30; or a combination of an enzyme, such as trypsin, and mechanical dissociation, as disclosed in Engelholm, S. A., M. Spang-Thomsen, et al. (1985). "Disaggregation of human solid tumours by combined mechanical and enzymatic methods." Br J Cancer 51(1): 93-8.

Lines 25 on page 21 to line 9 on page 22 have been amended as follows:

The active cells that have been concentrated, as described above, may be administered to a patient without further processing, or may be administered to a patient after being mixed with

other tissues or cells. In certain embodiments, the concentrated active cells (e.g., stem cells or endothelial precursor cells) are mixed with one or more units of adipose tissue that has not been similarly processed. Thus, by practicing the methods of the invention, a composition comprising adipose tissue with an enhanced concentration of active cells may be [administed] administered to the patient. The volumes of the various units of adipose tissue may be different. For example, one volume may be at least 25% greater than the volume of another unit of adipose tissue. Furthermore, one volume may be at least 50%, such as at least 100%, and even 150% or more greater than the volume of another unit of adipose tissue. In addition, the desired composition may be obtained by mixing a first unit of adipose tissue with the concentrated active cell population, which may be a cell pellet containing the active cells, with one or more other units of adipose tissue. In certain embodiments, these other units will not have an increased concentration of stem cells, or in other words, will have an active cell concentration less than that contained in the first unit of adipose tissue. In other embodiments, one of the units is cryopreserved material that contains, for example, an increased concentration of active cells.

Line 17 to line 27 on page 25 has been amended as follows:

By administering the stem cells and/or endothelial precursor cells to a patient, one can treat numerous diseases, including, and not limited to, bone-related disorders, diseases, or injuries, including slow/non-union fractures, osteoporosis (age-related or chemotherapy-induced), inherited diseases of bone (osteogenesis imperfecta); adipose related disorders or diseases; liver related diseases, disorders, or injuries, including liver failure, hepatitis B, and hepatitis C; myocardial infarctions, including heart attack or chronic heart failures; renal diseases or kidney damage; retinal diseases or_damage or necrosis; wound healing (e.g., from surgery or diabetic ulcers); skeletal muscle disorders both traumatic and inherited; [cartilege] cartilage and joint repair both traumatic and autoimmune; lung injuries; diabetes; intestinal disorders; nervous system disorders, [dieseases] diseases, or injuries, such as central nervous systems disorders, diseases, or injuries, including spinal cord injuries, Parkinson's disease, Alzheimer's disease, and stroke.

Line 9 to line 19 on page 28 has been amended as follows:

In certain embodiments, the component preparation chamber includes one or more ports for addition of agents that can enhance the process of separating stem cells for administering to a patient, such as growth factors or buffers for resuspending the cells, as discussed above. In these embodiments, component preparation chamber preferably includes a mixing device to mix or agitate the cells and additives in the container. Component preparation chamber also includes one or more ports for removing the cells collected therein. One port may be provided to pass the cells toward mixing container 30. Other ports may be provided to direct cells, or a portion of the cells, to other targets, such as implant materials, including bone fragments, or to cell culturing or purification devices. In one embodiment, the cell washing/separation chamber includes a spinning membrane filter component, which may be used as the cell concentrator in addition to or, preferably, [as] as an alternative to a centrifuge device.

Lines 30 on page 28 to line 27 on page 29 have been amended as follows:

In one embodiment, system 10 includes a temperature control device that is positioned with respect to system 10 to adjust the temperature of the material contained in the tissue collection container 12. In certain embodiments, the temperature control device is a heater, and in other embodiments, temperature control device is a cooler. In additional embodiments, the temperature control device may be able to switch between a heater and a cooler. The temperature control device may be a device that adjusts the temperature of the adipose tissue contained in tissue collecting container 12, or may be a device that is positioned to change the temperature of fluid being delivered to tissue collecting container 12. It has been found that heating the adipose tissue facilitates disaggregation of the tissue to enhance the separation of the active cell component. In addition, it is [desirabile] <u>desirable</u> in certain embodiments to cool a portion of the tissue, preferably the active cell component to provide protection to the cells. Even mild cooling of the cells may provide suitable protection to enhance cell survival during the processing.

Outlet 32 of tissue removal system 10 is illustrated as being a component of mixing container 30. In additional embodiments, outlet 32 is spaced apart from mixing container 30. Outlet 32 preferably comprises a closure that maintains the sealed configuration of tissue removal system 10, and in certain embodiments, outlet 32 comprises a fluid impermeable membrane (e.g., a membrane that is impermeable to liquid and air). Outlet 32 should be structured to pass the composition in mixing container 30 to a patient under the appropriate conditions. For example, if a syringe is used to withdraw the composition, outlet 32 should be able to accommodate a needle of the syringe without compromising the sterility of the system or composition. In additional embodiments, if the outlet is coupled to a device that is configured to administer the composition, but not to withdraw the composition, such as a cannula that administers the composition by applying positive pressure to displace the composition through the cannula, outlet 32 should be configured to allow the composition contained in mixing container 30 to be passed into the cannula. In other embodiments, outlet 32 may comprise, or be coupled in a closed-system fashion to, the device for [adminstering] administering the composition, such as a needle of a syringe or a cannula for administering the composition by applying positive pressure.

Line 2 to line 11 on page 31 has been amended as follows:

In order to reduce contamination within tissue removal system 10, one or more clamps 23 may be provided on the various lines or conduits to control the flow of material through the lines to the various components of the system. Clamps 23 permit a user to effectively seal various regions of tissue removal system 10. In a preferred embodiment, one or more of the components of system 10 are disposable. Avoiding reusing the components in this embodiment helps to reduce contamination that may be associated with repeated use of various components. In addition, providing the components in a disposable set provides an advantage of being able to sterilize all of the components at a single time, which may substantially reduce the time required for practicing the methods disclosed herein. In fully or partially [automoated] automated embodiments, computer-controlled valves may be implemented in addition to or as an alternative to clamps 23.

Line 19 to line 25 on page 31 has been amended as follows:

The components of the tissue removal system 10 should be made of materials that are non-reactive with biological fluids or tissues, and non-reactive with agents used in processing biological fluids and tissues. In [additon] addition, the materials from which the various components are made should be capable of withstanding sterilization, such as by autoclaving, and irradiation, including but not limited to beta- or gamma-irradiation. The tubing and the cannula handle may be made of any suitable material, such as polyethylene. The cannula may be made of stainless steel.

Lines 20 on page 38 to line 11 on page 39 have been amended as follows:

In additional embodiments of the invention, tissue collected into a conventional adipose tissue trap could be transferred into a processing set designed for processing other tissues. For example, Baxter Inc. manufacture and sell a series of plastic bags and filters intended for use in the setting of a bone marrow transplant harvest ("Bone Marrow Collection Kit with Flexible Pre-Filters and Inline Filters", Product Code, 4R2107, U.S. Pat. Nos. 4,346,703 and 5,724,988). This bag set contains a large conical bag with an integrated 800μm filter which could be used for washing the collected adipose tissue.[v] In this example adipose tissue fragments larger than 800μm would be retained in the bag. These fragments could then be washed by repeated addition of saline (or other washing solution) followed by removal of waste material through ports below the filter. Mixing could be achieved manually or by use of a benchtop rocking device and warming could be applied by use of a heating pad. Disaggregation could occur within the lumen of this bag. Following disaggregation cells would pass through the integrated 800μm filter (and optionally through one or more filters of smaller mesh size provided with the kit) and collected into a collection bag (also provided). This bag could then be placed into a centrifuge (e.g., a Sorval RC-3C) where cells could be serially washed and concentrated. Cells could also be washed using existing cell washing devices (largely developed for washing human blood products) such as those sold by Baxter Inc (Cytomate or Baxter CS3000) or by Cobe Inc.

(Cobe Spectra). The disposable elements may be integrated using the fittings provided by the manufacturer or they may be linked by use of a sterile connecting device such as those manufactured by Terumo Inc. Similarly the mechanisms described in this less integrated approach could be linked to a central controller and assembled as components of a more integrated device. A peristaltic pump or battery of pumps could be used to automate fluid flow with use of manual or automated clamping to open and close fluid pathways.

In the Claims:

Claims 1-66 and 80-92 have been canceled without prejudice.